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Review

Energy deficiency in the failing heart: Linking increased reactive oxygen species and disruption of oxidative phosphorylation rate

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Abstract

Heart failure is a complex syndrome of numerous dysfunctional components which converge to cause chronic progressive failure of ventricular contractile function and maintenance of cardiac output demand. The aim of this brief review is to highlight some of the mounting evidence indicating that augmented superoxide, related reactive oxygen species and other free radicals contribute to the oxidative stress evident during the progression of heart failure. While much of the source of increased reactive oxygen species is mitochondrial, there are other intracellular sources, which together are highly reactive with functional and structural cellular lipids and proteins. Bioenergetic defects limiting ATP synthesis in the failing myocardium relate not only to post-translational modification of electron transport respiratory chain proteins but also to perturbation of Krebs Cycle enzyme-dependent synthesis of NADH. Accumulation of pathological levels of lipid peroxides relate to dysfunction in the intrinsic capacity to clear and renew dysfunctional proteins. This review also features key limitations of human heart failure studies and potential clinical therapies that target the elevated oxidative stress that is a hallmark of human heart failure.

Keywords: Heart failure; Reactive oxygen species; Mitochondria; Krebs cycle enzyme; Antioxidant; Cardiolipin; Omega-3 PUFA

1. Introduction

Heart failure, a syndrome resulting in the chronic and progressive loss of ventricular systolic function, is one of the leading causes of death worldwide [1]. Despite significant advances in the treatment of heart failure, morbidity and mortality from heart failure remain high, with the overall one-year mortality estimated at 20–30% for mild to moderate failure and over 50% for severe heart failure. Heart failure may be categorised according to differing underlying etiology, including ischemic heart disease (coronary artery disease, myocardial infarction), cardiomyopathy (dilated, hypertrophic), valvular disease, alcohol/drug-induced heart disease and hypertensive heart disease. In economically advanced nations, ischemic heart disease accounts for almost 75% of heart failure cases [1].

Central to the loss of contractile function in heart failure is the inability of mitochondria to adequately supply the myocardium with ATP, resulting in energy deprivation to the cell and potentially necrotic/apoptotic cell death. It has been approximated that most mitochondrial ATP-derived energy supports myocardial contraction and the maintenance of ion homeostasis, 75% and 25% of cardiomyocyte energy consumption, respectively [2]. However, the underlying molecular causal events leading to metabolic dysfunction are poorly understood. The production of reactive oxygen species (ROS) has been shown to increase in the failing heart, and due to their close proximity to sites of superoxide production, mitochondrial proteins and lipids may be targets of oxidative damage in the failing heart. In this brief review, we describe the evidence linking increased mitochondrial ROS production in the failing myocardium and the potential for oxidative modification of mitochondrial proteins leading to dysfunction in ATP synthesis, and thus highlight potential targets for therapy.

2. Energy metabolism in heart failure: Krebs cycle versus respiratory chain

The heart is the greatest oxygen-consuming organ in the body, consuming around 8–15 ml O_2 min/100 g human heart

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tissue, with the capacity to increase to 70 ml under exercise conditions. [2]. Of this, 90% of available oxygen is consumed by mitochondria, which occupy over 30% of cardiomyocyte volume. Both fatty acid and carbohydrate metabolism converge at the Krebs cycle, with the generation of reduced redox carriers NADH and $FADH_2$ providing substrate for mitochondrial ATP synthesis. The Krebs cycle provides a major point of regulation of energy metabolism at three key enzymes, citrate synthase (CS), isocitrate dehydrogenase (ICDH) and α-ketoglutarate dehydrogenase (α-KGDH) due to their highly negative $\Delta G^{\circ\prime}$ values (standard free energy of ATP hydrolysis) [3]. Notably, the latter two of these three enzymes, along with pyruvate dehydrogenase, are linked to complex I via their supply of NADH, and thus serve NADH oxidation and the regulation of energy flow through the electron transport chain protein complexes, resulting in the reduction of oxygen to water. The proton motive force generated by this process drives ATP synthesis via the F_1Fo–ATP synthase complex (complex V) [3].

Reduced ATP synthesis, as measured by a lowering of state III (ADP-coupled) respiration has been demonstrated in isolated cardiac mitochondria from failing animal [4] and human [5] hearts, relative to non-failing controls. In an attempt to identify possible causes for this decline, many studies have focused on the measurement of the electron transport chain enzymes in a number of human cardiomyopathies. Most notably, complex I [6], complex III [7], and complex IV [8] have been identified as dysfunctional in end-stage human heart failure. A direct causal relationship, however, has proven elusive over the years, with a poor correlation between reduced complex activities and the severity of disease [7]. This may be partly explained by a large reserve capacity for activity of each respiratory protein complex. For example, up to 50% inhibition of complex I or IV activity is required before a significant decline in state III respiration is apparent [9].

In contrast, Krebs cycle enzymes involved in the regulation of substrate metabolism appear to exert more direct control over energy output, with a close correlation between the loss of α-KGDH activity and decreased state III respiration [13]. Due to their close interaction with complex I, a major site of mitochondrial superoxide formation, NADH-linked enzymes such as α-KGDH and ICDH may be susceptible to ROS-dependent perturbation. Reduced ICDH activity (~30%) has been demonstrated as an early marker of hypertrophy before the onset of ventricular dysfunction, in transgenic hypertrophic cardiomyopathic mice [10] and spontaneously hypertensive rats (SHR), [11]. The decline in α-KGDH, PDH and ICDH activity coincides with the formation of protein thiol adducts with 4-hydroxy-2-nonenal (HNE), a lipid peroxidation aldehyde formed via reaction between arachidonic acid and superoxide [12–16]. Reduced α-KGDH activity together with increased HNE-adduct formation has also been described in aged rats [9]. However, the specific role that perturbation of these enzymes may play in contributing to an energy deficit in the failing heart awaits detailed examination. Nevertheless it is apparent that diminished mitochondrial energy metabolism in the failing heart involves dysfunction in Krebs cycle regulation, NADH supply and activity of electron transport chain proteins.

3. Heart failure is a state of augmented oxidative stress

There is a large body of evidence that markers of oxidative stress are augmented in the plasma and tissue of patients with heart failure [17,18]. Due to the short half-life of oxygen free radicals, most studies have relied on the measurement of lipid oxidation products. However, these products (such as malondialdehyde, MDA) are often removed from the source and magnitude of the initial oxidative reaction, as they are intermediates that may undergo further conversion or reaction with proteins, and thus offer limited diagnostic marker specificity. Despite this, the convenience of assay simplicity, low cost and general association with oxidation rate has sustained their continued use. Elevated MDA concentration has been measured in the plasma of patients with moderate symptoms of congestive heart failure (NYHA III, left ventricular ejection fractions less than 40%), compared to age-matched non-failing controls (ejection fractions greater than 40%), which increased with the duration (years) of congestive heart failure [17]. A significantly high concentration of plasma MDA and reduced thiol has also been reported in heart failure patients with underlying coronary artery disease [19]. Mak and coworkers [20] demonstrated that total aldehydes are elevated in the plasma of heart failure patients, with a strong negative correlation between total aldehydes and contractility (+dP/dt) as well as increased time to relaxation (−dP/dt). Keith et al. [18] also reported a correlation between severity of heart failure and elevated lipid peroxides and MDA in both ischemic heart disease and dilated cardiomyopathy patients with end-stage heart failure. Another indirect marker of oxidative stress, 8-*iso*-prostaglandin $F_2\alpha$, derived from the oxidation of arachidonic acid, is increased in the pericardial fluid of patients in proportion to the severity of heart failure of ischemic and/or valvular disease origin [21].

Direct evidence of augmented myocardial superoxide production in the failing heart has been established in studies using electroparamagnetic spin resonance (EPR) spectroscopy ("spin-trapping"). Increased superoxide production has been demonstrated in the myocardium of explanted human heart failure tissue compared to non-failing donor heart muscle [22], confirming the results obtained in a pacing-induced animal model of heart failure [23]. However, in vivo, real time demonstration of increased cardiac superoxide metabolism in human heart failure is yet to be determined. It is also crucial to determine whether there are differences in superoxide metabolism according to the etiology, responsible cell type (smooth muscle, fibroblast, endothelial cell or cardiomyocyte) and progression of human heart failure.

4. Sources of oxygen radicals in the failing myocardium

A significant contribution of oxygen radical generation in the cardiomyocyte originates within mitochondria during oxidative phosphorylation. It is estimated that 1–2% of oxygen is incompletely converted to H_2O during electron transfer, resulting in the formation of ROS such as the superoxide ($-O_2^-$) and hydroxyl (OH^-) anions and hydrogen peroxide (H_2O_2) [24]. The reactivity of oxygen derives from its incomplete pairing of

electrons in the outer electron shell, and thus single electron transfer forms the superoxide anion, $-O_2^-$ [3]. The proposed sites of mitochondrial superoxide formation are at complex I (NADH dehydrogenase) [25], and the ubisemiquinone site of complex III [26], as demonstrated using targeted inhibitors. Due to the location of sites of oxygen radical formation, complex I is expected to release superoxide towards the mitochondrial matrix, whereas superoxide generated at complex III may be directed towards both the matrix and intermembrane space [26,27]. Through the use of mass spectroscopy, protein subunits protruding into the mitochondrial matrix have been shown to be most at risk from oxidative damage as targets of HNE binding, or carbonyl and nitrosyl modification under basal conditions [28]. Increased superoxide formation by complex I has been directly measured in the sub-mitochondrial fraction from failing cardiomyocytes, together with a significant loss of complex I activity [23], implicating the mitochondrial electron transport chain as a significant contributor to the pathogenesis of heart failure.

It has been proposed that the amount of superoxide formed is increased under state IV as opposed to state III respiration, due to lower ADP availability and increased molecular oxygen, whereas uncoupling conditions lower the production of ROS. Basal respiration has been shown to increase in patients with congestive heart failure [30] by increased resting oxygen consumption, indicating an underlying increase in basal ROS production in the diseased state. Under conditions of inefficient mitochondrial respiration due to reduced complex I activity, ROS production may also be increased [31,32].

5. Endogenous antioxidant systems and ROS management

Mitochondria possess a number of endogenous antioxidant systems which normally metabolise ROS under physiological conditions. These include enzymes such as manganese superoxide dismutase (MnSOD), glutathione peroxidase and catalase, as well as non-enzymatic components such as coenzyme Q_{10}, glutathione, α-lipoic acid and vitamin E. Although it is beyond the scope of this review to specifically cover each of these, it is important to note that under normal conditions they constitute a complex network of redox-signal transduction and ROS management essential to the containment of ROS-mediated damage but in heart failure their capacity is breached. MnSOD, which is exclusively mitochondrial, accounts for 70% of total heart SOD activity and 90% of that within the myocyte. MnSOD converts superoxide to hydrogen peroxide, which in turn is converted to water by catalase and glutathione peroxidase, which uses reduced glutathione as an electron donor. However, under certain circumstances, H_2O_2 may react with free iron or copper to produce the highly aggressive OH^- radical [31]. The crucial nature of MnSOD was illustrated experimentally when MnSOD expression was inhibited in a mouse knockout model. Early death was prevalent for these mice as a result of dilated cardiomyopathy that occurred with a concomitant rise in ROS production [29]. Whether antioxidant status directly contributes to the development or severity of heart failure remains under debate. However, in diseased tissue where increased mitochondrial superoxide production together with reduced antioxidant capacity may exist, the possibility of overproduction of oxygen free radicals and hence damage to mitochondrial components may therefore increase. The impact of this effect on cardiac cells is exacerbated when ROS production at non-mitochondrial sites is also augmented.

6. Non-mitochondrial sources of ROS in the failing heart

ROS are also produced in the heart through several other cellular processes. These include the enzymatic xanthine–xanthine oxidase system and NAD(P)H oxidase, activation of inflammatory pathways by infiltrating granulocytes (neutrophils and monocytes), cytochrome P450, the oxidation of catecholamines, as well as the uncoupling of nitric oxide (NO) synthase (NOS) [3,17,33]. Under certain conditions, NO may also pair with superoxide to form highly reactive peroxynitrite, however the source of NO may also be mitochondrial [34–37].

In human and animal models of heart failure, xanthine oxidase expression is markedly augmented [38–40]. A recent study examining a canine model of pacing-induced heart failure [41] showed that treatment of failing hearts with allopurinol or ascorbic acid resulted in increased contractility and improved mechanical efficiency on the basis of increased work performed per unit of oxygen. However, this amelioration of mechanical work-oxygen utilisation efficiency could be prevented with the NOS inhibitor, N^G-monomethyl-L-arginine. In non-failing control hearts, similar NOS inhibition resulted in diminished mechanical efficiency that could be reversed by allopurinol or ascorbic acid. Together these data indicate a close interaction between the xanthine oxidase and NOS systems over their regulation of oxygen metabolism and myocardial contractile function and which also underlie mechano-energetic uncoupling in the failing heart.

The primary function of NAD(P)H oxidases appears to be ROS production via the electron transfer of NADPH to molecular oxygen [42]. While there are 5 isoforms of NAD(P)H oxidases, each encoded by separate genes, these have varied expression according to cell type, with isoforms 2 and 4 being most abundantly expressed in endothelial cells, fibroblasts and myocytes [42]. Heymes and coworkers [43], reported in a recent study that myocardial NAD(P)H oxidase activity and related ROS formation was increased in end-stage heart failure patients and was related predominantly to altered translocation of the p47 regulatory subunit of NAD(P)H oxidase. Pathophysiological features common in the development of cardiac hypertrophy and heart failure that have been shown to also trigger activation of NAD(P)H oxidases include, Angiotensin II [44], endothelin 1 [45], α-adrenergic receptor activation [46], TNF-α, P38 MAPK [33,37,47–49], and stretch [50]. Secondary consequences of NAD(P)H oxidase-derived ROS may involve subsequent promotion of further ROS formation, in particular this includes the activation of xanthine oxidase [51], and the oxidation of the essential NOS cofactor tetrahydrobiopterin that results in the uncoupling of NOS [52]. Such consequences may be intrinsic to facilitating redox-sensitive intracellular signal transduction involved in the adaptive remodelling mechanisms that included fibroblast and vascular smooth muscle cell proliferation, myocyte hypertrophy, fibrosis and alteration of extracellular

matrix, during the development of hypertrophy and early heart failure [53–56]. However the specific pathways and extent of signalling pertaining directly to NAD(P)H oxidase activation are yet to be fully delineated.

7. The impact of ROS on NO regulation of mitochondrial function

As described above, ROS-induced oxidation of tetrahydrobiopterin results in the uncoupling of NOS [52], thus abrogating the physiological regulatory role of NOS over mitochondrial function and subsequently yielding to the increased expression of NOS isoforms involved in pathological sequelae. NO is formed via NOS from the substrates arginine, oxygen and NADPH and obligatorily requiring tetrahydrobiopterin and calmodulin [57]. Although there are three NOS isoforms dependent on separate genes; endothelial NOS, neuronal NOS, and inducible NOS [58], mitochondrial NOS has also been reported [59–61]. However it remains unclear as to whether mitochondrial NOS is derived from one of the three reported NOS isoforms, particularly as NO easily and rapidly diffuses in water and through membranes. NOS isoforms are heterogeneously distributed not only in the numerous cell types of the heart but also across subcellular compartments, for example, in myocytes NOS isoforms are expressed in the sarcolemma, sarcoplasmic reticulum, and mitochondria [61–63]. Under physiological conditions, NO nitrosylates the haem moiety of soluble guanylyl cyclase to activate the formation of 3′,5′-cyclic guanosine monophosphate and subsequently activate protein kinase G in a complex cascade of signal transduction underlying numerous regulatory events [64]. NO from endothelial NOS plays a crucial cardiac role in coronary vasodilation, chronotropy, atrioventricular node function, inotropy, neutrophil and platelet regulation, it also regulates mitochondrial oxygen consumption [65–69]. At nanomolar concentrations NO also regulates mitochondrial respiration by reversibly competing with oxygen for the cytochrome *c* oxidase (complex IV) oxygen binding site on a beat-to-beat, cyclic basis, and despite reducing oxygen consumption, actually improves mechanical efficiency (stroke work relative to oxygen utilisation) and permits oxygen consumption to efficiently meet fluctuations in oxygen supply [41,61,70–72].

Poderoso et al. [73] demonstrated in isolated rat mitochondria that inhibition of the respiratory chain by physiological levels of NO enhances superoxide formation which reacts with NO to form peroxynitrite anion and thus in doing so permits reversal of NO-induced inhibition and resumption of cytochrome *c* oxidase activity. Under these physiological conditions intramitochondrial peroxynitrite levels are low and extremely short lived (<1 s half-life) and only when peroxynitrite is elevated to high micromolar concentrations that succinate dehydrogenase is inhibited and H_2O_2 formation augmented [74]. Thus, it has been proposed that this NO-dependent reversible inhibition of cytochrome *c* oxidase is a mechanism that facilitates oxygen diffusion further along its gradient attenuating the steepness of the PO_2 gradient across to more distant mitochondria and cells [73].

In contrast, inducible NOS increases NO to supra-physiological levels (μM) in response to inflammatory cytokines, shear stress and other conditions often linked to, but not exclusive to, cardiac pathology [75]. Peroxynitrite and related reactive nitrite species, formed when NO reacts with superoxide, promote inhibition of mitochondrial respiratory proteins and other proximal proteins such as catalase, aconitase, superoxide dismutase and creatine kinase via lipid peroxidation and protein thiol nitrosylation [70,76–78]. Under physiological conditions, glutathione-dependent formaldehyde dehydrogenase has been identified in its capacity to degrade sulphide–NO bonds, however this has not been well characterised in the heart [79,80]. It is yet to be determined whether there are the specific changes in the expression or function of this enzyme system during the development of heart failure.

Notably NO plays an important role in anti- and pro-apoptotic signal transduction. While low nanomolar amounts of NO keep the mitochondrial permeability transition pore closed, high micromolar levels promote pore opening and cytochrome *c* release eventually triggering apoptosis when sustained [81,82]. In chronic heart failure, NO and TNFα production are markedly augmented in proportion to severity of failure [83]. NO has been demonstrated to increase Bax protein expression and initiate caspase activation facilitating apoptosis [84,85]. In post-infarct models of heart failure, apoptosis contributes to poor cell survival of myocytes in the remote myocardium and in cardiac allograft rejection [86–91]. Thus, in heart failure, increased NO and superoxide formation, increased NO inhibition of respiration, increased peroxynitrite and reactive nitrite species formation, increased lipid peroxidation and thiol/protein adduct formation ultimately lead to the inactivation of numerous mitochondrial proteins. With mitochondrial function greatly compromised, subsequent energy dissipation and contractile dysfunction contribute further to the spiralling progression of cellular injury and heart failure.

8. Indirect markers of ROS-dependent cell damage

The superoxide anion is relatively short-lived and impermeable to the mitochondrial inner membrane, making its direct measurement difficult. Experimental measures have therefore relied on the use of secondary markers of ROS formation. Unsaturated membrane fatty acids are a particular target of oxygen radical attack due to their unsaturated bonds, resulting in the formation of lipid peroxidation products. Aldehydic products of high biological abundance are malondialdehyde (MDA, saturated) and 4-hydroxy-nonenal (HNE, unsaturated). HNE is formed by the interaction between membrane omega-6 polyunsaturated fatty acids and superoxide, producing a relatively stable, highly unsaturated aldehydic compound. Compared to the limited action of ROS, the stability of aldehydes such as HNE readily permits diffusion from the site of initial ROS generation and produce protein perturbation distant from their origin, thus propagating and amplifying the oxidative injury. Due to its unsaturated bonds, HNE readily attacks amino acid residues, primarily cysteine, lysine and histidine residues through Michael-type reactions and has been shown to cause

extensive protein cross-linking, disruption of mitochondrial gene transcription and is generally a potent 'toxic' second messenger pervading numerous other intracellular targets [12,92]. HNE has been show to be elevated in the plasma of heart failure patients [20], and has been demonstrated as a potent inhibitor of mitochondrial NADH-linked state III respiration in isolated rat heart mitochondria at pathological concentrations [13–15]. Aldehydes, including HNE, are detoxified and cleared via the aldose reductase pathway [93], recently identified as an important component of the heart's intrinsic capacity to manage lipid peroxidation-derived aldehydic products under physiological conditions. It has thus been hypothesised that the accumulation of lipid peroxides in plasma and myocardium of heart failure patients may, at least in part, be related to dysfunction of the aldose reductase pathway. Srivastava and colleagues [93], recently found support for this proposal using a chronic canine model of left ventricular-pacing induced heart failure. In early and late stages of heart failure, oxidative stress indices increased while cardiac aldose reductase expression and activity decreased progressively during the progression of heart failure. In early heart failure translational and/or post-translational modification of aldose reductase occurs, whereas in the late stages of heart failure there is a reduced transcriptional regulation and expression of aldose reductase [93]. However, the direct measures, specific role and the mechanisms of HNE contribution to mitochondrial dysfunction in human heart failure have yet to be examined.

9. ROS damage to mitochondria

Increased oxidative stress in the failing myocardium is associated with global cellular defects, including impaired contractility and electrical instability [19]. Cellular targets of oxidative attack include the sarcoplasmic reticulum Ca^{2+} pump, resulting in reduced Ca^{2+}efflux, ultimately increasing likelihood for Ca^{2+}-overload [94]. HNE impairment of other ion handling proteins such as the Na^{+}/K^{+}ATPases [95,96] and Ca^{2+}–Mg^{2+}ATPases [97] has been demonstrated in vitro.

Exposure to free radicals in the intact ventricles of rabbits and guinea pigs has been shown to cause rapid increase in K^{+} efflux, as well as progressive shortening of the action potential, with a 50% reduction in tissue high-energy phosphates (ATP and CP) [98]. In isolated patch-clamped myocytes, free-radical generation (xanthine–xanthine oxidase) resulted in irreversible inhibition of glycolytic and mitochondrial oxidative metabolism, matched to that induced by FCCP and the glycolytic inhibitor 2-DOG [98].

Much of the work involving oxygen radical damage to mitochondria has focused on ischemia–reperfusion injury. Following an acute ischemic insult and reperfusion of the hypoxic myocardium, a burst of ROS is seen within the first few minutes [99,100], resulting from recommencement of mitochondrial oxidative phosphorylation. Due to the low ADP levels in ischemic tissue, when oxidative phosphorylation recommences, lack of ADP means that electron transfer is impaired, resulting in a build up of incomplete oxygen molecules. Of particular susceptibility to mitochondrial oxidative damage are membrane-bound proteins, lipids and DNA in close proximity to the sites of superoxide formation, namely the electron transport chain complexes I, III and IV, unsaturated membrane phospholipids and mitochondrial DNA (mtDNA). As discussed in the sections above, the reaction of superoxide with NO initiates the formation of peroxynitrite and related reactive nitrite species which also inhibit the activity of numerous mitochondrial proteins and accentuate lipid peroxidation and further ROS formation [70,76–78]. It is, however, important to now draw our attention to the impact of oxidative damage on cardiolipin because the loss of cardiolipin has marked consequences on mitochondrial function [101,102].

Cardiolipin, due to its highly unsaturated fatty acid chains, is particularly susceptible to oxidative attack [103–105]. Due to its close interaction with sites of ROS formation (complexes I and III) it is a strong target of oxidative attack in the reperfused myocardium. Paradies and associates have reported 25% and 50% reductions in activities of complexes I and III following ischemia and ischemia–reperfusion, with significant increases in mitochondrial MDA levels [103,104]. Complex IV, which requires a tight association with cardiolipin for optimal function was also reduced to the same extent following IR, despite constant amounts of heme aa3 being present in control and ischemic groups [105]. Reperfusion also resulted in a significant decrease in mitochondrial membrane cardiolipin content.

Cardiolipin has been recognized as being an essential factor for the correct assembly and function of electron transport chain complexes and other components of the inner mitochondrial membrane and is required for their optimal function [106]. Interestingly, cardiolipin treatment of mitochondria isolated from hearts subjected to ischemia–reperfusion restored electron transport complex activities to almost control levels, thus demonstrating a major role of cardiolipin in the overall function of the mitochondrion [107].

In addition to complexes I, III and IV, the adenine translocase has been demonstrated as having an absolute requirement for cardiolipin and is highly susceptible to ROS [107]. Notably, perturbation or loss of cardiolipin is a key contributing factor to mitochondrial release of cytochrome *c* and apoptosis [106,108]. Although there is some evidence of diminished cardiolipin levels in heart failure from animal models [109,110], no published studies to date have directly measured cardiolipin levels in the failing human heart.

Unlike nuclear DNA, mitochondrial DNA is more susceptible to oxidative attack, lacking introns, histones and other DNA-associated proteins involved in protection or faster rates of DNA repair. Under basal conditions, mitochondrial DNA displays increased oxidative damage to guanosine bases compared to nuclear DNA, indicating the likelihood of accumulated mtDNA damage leading to disease is therefore higher [111]. In a post-myocardial infarcted murine model of heart failure, increased superoxide production coinciding with decreased mtDNA copy number has been demonstrated. Gene expression of the electron transport chain subunits encoded on the mitochondrial genome, whose transcription is governed by the mtDNA copy number, was also reduced, reflected by reductions in activity of complexes I, III and IV [112]. However, in end-

stage failing human tissue, this does not appear to be the case, as in both ischemic and dilated cardiomyopathic human explant tissues, no alteration in gene expression has been detected in any of the mitochondrial-encoded electron transport chain subunit genes [6,7]. This suggests that disruption of enzymes involved in mitochondrial energy metabolism may occur at the post-translational level.

10. Antioxidant and other therapy in heart failure

The issue of whether antioxidant therapy is an effective treatment for congestive heart failure remains in heated debate. Coenzyme Q_{10}, which is an essential component of electron transfer as well as a potent antioxidant in all cell membranes, has been the subject of numerous human trials. While many received positive results in improvements in contractility and blood pressure following treatment, most showed no overall difference in patient mortality, within the limitations of study design. Coenzyme Q_{10} treatment outcome effects in human trials have been mixed [113–115].

Improvements in terms of reduced oxidative stress (TBARS) have also been reported with administration of vitamin E, and this was associated with improved cardiac contractility [116]. In addition, a 20% reduction in mortality at 20 weeks was seen in the vitamin E-treated heart failure rats compared to sham controls, as well as reduced clinical heart failure symptoms (dyspnea, abdominal enlargement, limb cyanosis). However the use of vitamin E is controversial in humans and is associated with negative outcome studies and numerous concerns [117].

New considerations of the use of omega-3 polyunsaturated fatty acids in heart failure are presently being tested clinically [118]. The impetus for this therapeutic strategy is the mounting evidence of the pervasive beneficial impact of omega-3 polyunsaturated fatty acids on mitochondrial biogenesis, nuclear receptor-dependent changes in transcription, intrinsic antioxidant enzyme expression, eicosanoid and cytokine signalling, membrane and cytosolic/matrix protein function (ion channels, pumps, enzymes) and efficiency in oxygen consumption [119–122].

One problem faced by even the most potent of agents, is that once the pattern of cell loss and cardiac remodelling is in place, late commencement of therapeutic treatment may only limit further damage, and advanced progression to severe end-stage failure may prevent the myocardium to survive and sustain function. New therapies must not only treat hemodynamic dysfunction and symptoms associated with heart failure, but also slow the initiation of cell loss and oxidative damage [123]. Agents such as Levosimendan have been proven to be promising in both the short-term treatment and long-term survival of patients with acute heart failure. Levosimendan enhances contraction by increasing myofilament sensitivity to calcium [124,125]. In addition, Levosimendan alters cardiac metabolism by its potent vascular smooth muscle vasodilation via activation of K_{ATP} channels in the plasma membrane, and by also acting directly at mitochondrial K_{ATP} channels (see review by Papp [126]). Maytin and coworkers [123], have demonstrated that incubation of isolated rabbit left ventricular myocytes in culture with levosimendan reduces hydrogen peroxide-induced apoptosis to approximately half that of control (hydrogen peroxide only), which could be blocked by the K_{ATP} channel blocker 5-HD. In addition to improving hemodynamic function, it also results in a significant improvement in long-term survival [127].

Other drugs also exert antioxidant or free radical scavenging effects in addition to their effects on vasodilation and beta-adrenergic receptor blockade. Hydralazine, a very old vasodilator drug, has in recent times been used successfully to improve cardiac output, reduce cardiac filling pressures, improve ejection

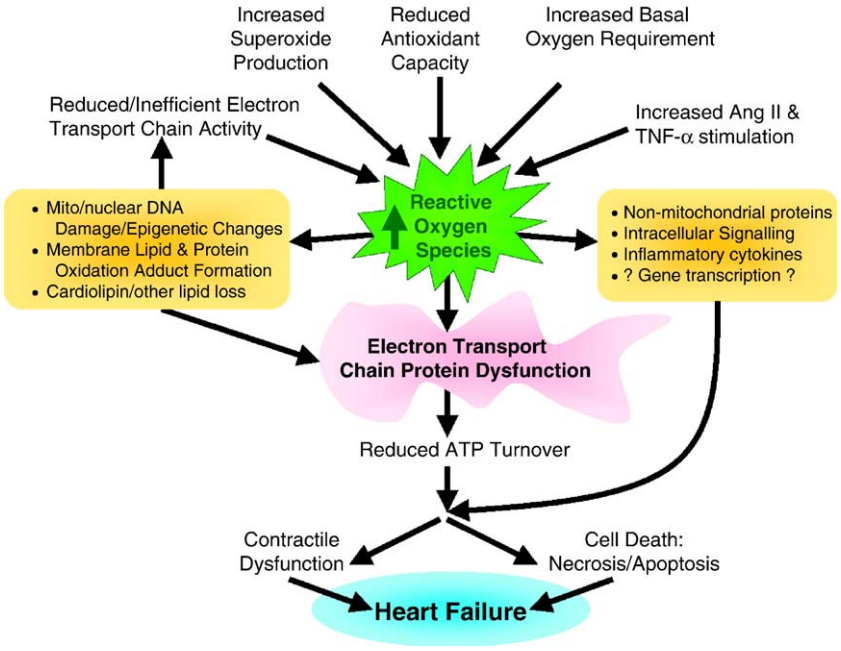

Fig. 1. Scheme of factors influencing mitochondrial ROS production in the failing myocardium and the effects of oxygen radical damage on cellular dysfunction and death.

fractions and augment exercise capacity in afterload-dependent patients with congestive heart failure [128]. In particular, hydralazine is able to limit nitrate tolerance that blunts the anti-ischemic effects of glyceryltrinitrate and is a potent scavenger of free radicals that limits peroxynitrite formation [36]. While nicorandil beneficially opens both K_{ATP} channels and donates nitric oxide, it also limits superoxide-dependent formation of urinary 8-epi-prostaglandin-F2α in patients with acute myocardial infarction [129]. Carvedilol was used initially for its beta-adrenergic receptor blockade action. Most notably, carvedilol treatment of patients with dilated cardiomyopathy for 9 months, not only resulted in amelioration of heart failure symptoms but also reduced HNE-protein adduct immuno-reactivity in ventricular tissue biopsies by 40% [130]. Despite encouraging promise from many potential therapeutic agents, considerable effort is still required to convincingly demonstrate efficacy in human heart failure at clinical trial level. The complexity of the multiple defects, adaptations, diverse etiology and rates of progression that occur in human heart failure, coupled with the expense and difficulty of executing clinical trials, is a major hurdle to overcome before new therapeutic agents are routinely employed successfully.

11. Limitations of human studies and conclusion

The study of oxidative stress-induced mitochondrial damage in human heart diseased tissue has been problematic. One of the main problems faced is obtaining true controls. Normal (non-failing) tissue is often collected up to 6 h post-mortem, being subjected to brain death and ischemic stress up to this point. In some cases 'control tissue' may in fact contain more oxidative damage than the failing group. Many of these mitochondrial defects in particular are also subject to age-dependent differences. ROS production is known to increase with age, as does reduced antioxidant capacity. These factors need to be taken into account before final analyses. Unlike most animal heart failure models, human tissue studies are also complicated by prior heart failure treatments. As most published biochemical studies have relied on explanted severe end-stage failing hearts, the data acquired in these studies may not necessarily be extrapolated back to patients that are in earlier, more moderate stages of heart failure. As discussed above some drugs currently used in heart failure also have antioxidant properties, and many patients today self-treat with non-pharmaceutical agents such as coenzyme Q_{10} and vitamin E. Consumption of these may be masking true ROS levels. Finally, most of the sampling of ROS has involved downstream markers such as lipid peroxidation products, and often sampling has been only in plasma, thus not reflecting tissue-specific ROS metabolism.

Despite these limitations, there is certainly evidence that oxidative stress is increased in the failing myocardium and that amongst many factors, key mitochondrial proteins (respiratory chain and Krebs Cycle) at close proximity to the sites of ROS generation are subsequently perturbed, limiting or ceasing function. (See Fig. 1 for a general scheme summary). Whether oxidation-induced damage plays a significant role in dysfunction in mitochondrial oxidative phosphorylation in human heart failure warrants further investigation. New agents and clinical trials are required to determine whether specific treatments that directly target the efficiency of oxygen metabolism, NADH and ATP synthesis, ROS formation and handling, and the clearance of lipid and protein adducts from cell membranes, plasma and myocyte contractile components, may prove valuable in attenuating the progression of heart failure.

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References

[1] B.S.P. Chin, M.K. Davies, G.Y.H. Lip, Heart Failure in Practice, Royal Society of Medicine, London, 2002.
[2] F.J. Giordano, Oxygen, oxidative stress, hypoxia and heart failure, J. Clin. Invest. 115 (2005) 500–508.
[3] R.G. Hansford, Control of mitochondrial substrate oxidation, Curr. Top. Bioenerg. 10 (1980) 217–277.
[4] V.G. Sharov, A. Goussev, M. Lesch, S. Goldstein, H.N. Sabbah, Abnormal mitochondrial function in myocardium of dogs with chronic heart failure, J. Mol. Cell. Cardiol. 30 (1998) 1757–1762.
[5] V.G. Sharov, A.V. Todor, N. Silverman, S. Goldstein, H.N. Sabbah, Abnormal mitochondrial respiration in failed human myocardium, J. Mol. Cell. Cardiol. 32 (2000) 2361–2367.
[6] R.J. Scheubel, M. Tostlebe, A. Simm, S. Rohrbach, R. Prondzinsky, F.N. Gellerich, R.-E. Silber, J. Holtz, Dysfunction of mitochondrial respiratory chain complex I in human failing myocardium is not due to disturbed mitochondrial gene expression, J. Am. Coll. Cardiol. 40 (2002) 2174–2181.
[7] D. Jarreta, J. Orus, A. Barrientos, O. Miro, E. Roig, M. Heras, C.T. Moraes, F. Cardellach, J. Cascademont, Mitochondrial function in heart muscle from patients with idiopathic dilated cardiomyopathy, Cardiovasc. Res. 45 (2000) 860–865.
[8] E. Arbustini, M. Diegoli, R. Fasani, M. Grasso, P. Morbini, N. Banchieri, O. Bellini, B. Dal Bello, A. Pilotto, G. Magrini, C. Campana, P. Fortina, A. Gavazzi, J. Narula, M. Vigano, Mitochondrial DNA mutations and mitochondrial abnormalities in dilated cardiomyopathy, Am. J. Pathol. 153 (1998) 1501–1510.
[9] D.T. Lucas, L.I. Szweda, Declines in mitochondrial respiration during cardiac reperfusion: age-dependent inactivation of alpha-ketoglutarate dehydrogenase, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 6689–6693.
[10] D.T. Lucas, P. Aryal, L.I. Szweda, W.J. Koch, L.A. Leinwand, Alterations in mitochondrial function in a mouse model of hypertrophic cardiomyopathy, Am. J. Physiol. 284 (2003) H575–H583.
[11] M. Benderdour, G. Charron, D. de Blois, B. Comte, C. Des Rosiers, Cardiac mitochondrial NADP+-isocitrate dehydrogenase is inactivated through 4-hydroxynonenal adduct formation, J. Biol. Chem. 278 (2003) 45154–45159.
[12] H. Esterbauer, R.J. Schaur, H. Zollner, Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes, Free Radic. Biol. Med. 11 (1991) 81–128.
[13] K.M. Humphries, Y. Yoo, L.I. Szweda, Inhibition of NADH-linked mitochondrial respiration by 4-Hydroxy-2-nonenal, Biochemistry 37 (1998) 552–557.
[14] K.M. Humphries, L.I. Szweda, Selective inactivation of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase: reaction of lipoic acid 4-hydroxy-2-nonenal, Biochemistry 37 (1998) 15835–15841.
[15] H.A. Sadek, K.M. Humphries, P.A. Szweda, L.I. Szweda, Selective inactivation of redox-sensitive mitochondrial enzymes during cardiac reperfusion, Arch. Biochem. Biophys. 406 (2002) 222–228.

[16] M. Benderdour, G. Charron, B. Comte, R. Ayoub, D. Beaudry, S. Foisy, D. de Blois, C. Des Rosiers, Decreased mitochondrial NADP+-isocitrate dehydrogenase activity and expression: a marker of oxidative stress in hypertropy development, Am. J. Physiol. 287 (2004) 2122–2131.
[17] C.R. Diaz-Velez, S.G. Castineiras, E. Mendoza-Ramos, E. Hernandez-Lopez, Increased malondialdehyde in peripheral blood of patients with congestive heart failure, Am. Heart J. 131 (1996) 146–152.
[18] M. Keith, A. Geranmayegan, M.J. Sole, R. Kurian, A. Robinson, A.S. Omran, Increased oxidative stress in patients with congestive heart failure, J. Am. Coll. Cardiol. 31 (1998) 1352–1356.
[19] J. McMurray, J. McLay, M. Chopra, A. Bridges, J.J.F. Belch, Evidence for enhanced free radical activity in chronic congestive heart failure secondary to coronary artery disease, Am. J. Cardiol. 65 (1990) 1261–1262.
[20] S. Mak, D.C. Lehotay, M. Yazdanpanah, E.R. Azevedo, P.P. Liu, G.E. Newton, Unsaturated aldehydes including 4-OH-nonenal are elevated in patients with congestive heart failure, J. Card. Fail. 6 (2000) 108–114.
[21] Z. Mallat, I. Phillip, M. Lebret, et al., Elevated levels of 8-*iso*-prostaglandin F2a in pericardial fluid of patients with heart failure: a potential role for in vivo oxidant stress in ventricular dilatation and progression to heart failure, Circulation 97 (1998) 1536–1539.
[22] F. Sam, D.L. Kerstetter, D.R. Pimental, S. Mulukutla, A. Tabaee, M.R. Bristow, W.S. Colucci, D.B. Sawyer, Increased reactive oxygen species production and functional alterations in antioxidant enzymes in human failing myocardium, J. Card. Fail. 11 (2005) 473–480.
[23] T. Ide, H. Tsutsui, S. Kinugawa, H. Utsumi, D. Kang, N. Hattori, K. Uchida, K.-I. Arimura, K. Egashira, A. Takeshita, Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium, Circ. Res. 85 (1999) 857–863.
[24] J.F. Turrens, Superoxide production by the mitochondrial respiratory chain, Biosci. Rep. 17 (1997) 3–8.
[25] J.F. Turrens, A. Boveris, Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria, Biochem. J. 191 (1980) 421–427.
[26] J.F. Turrens, A. Alexandre, A.L. Lehninger, Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria, Arch. Biochem. Biophys. 237 (1985) 408–414.
[27] E.J. Lesnefsky, S. Moghaddas, B. Tandler, J. Kerner, C.L. Hoppel, Mitochondrial dysfunction in cardiac disease: ischemia–reperfusion, aging and heart failure, J. Mol. Cell. Cardiol. 33 (2001) 1065–1089.
[28] K.B. Choksi, W.H. Boylston, J.P. Rabek, W.R. Widger, J. Papaconstantinou, Oxidatively damaged proteins of heart mitochondrial electron transport complexes, Biochim. Biophys. Acta 1688 (2004) 95–101.
[29] Y. Li, T.T. Huang, E.J. Carlson, S. Melov, P.C. Ursell, J.L. Olson, L.J. Noble, M.P. Yoshimura, C. Berger, P.H. Chan, D.C. Wallace, C.J. Epstein, Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase, Nat. Genet. 11 (1995) 376–381.
[30] E.T. Poehlman, J. Scheffers, S.S. Gottlieb, M.L. Fisher, P. Vaitekevicius, Increased resting metabolic rate in patients with congestive heart failure, Ann. Intern. Med. 121 (1994) 860–862.
[31] S. Pitkanen, B.H. Robinson, Mitochondrial complex I deficiency leads to increased production of superoxide radicals and induction of superoxide dismutase, J. Clin. Invest. 98 (1996) 345–351.
[32] X. Luo, S. Pitkanen, S. Kassovska-Bratinova, B.H. Robinson, D.C. Lehotay, Excessive formation of hydroxyl radicals and aldehydic lipid peroxidation products in cultured skin fibroblasts from patients with complex I deficiency, J. Clin. Invest. 99 (1997) 2882–2887.
[33] D. Sorescu, K.K. Griendling, Reactive oxygen species, mitochondria, and NAD(P)H oxidases in the development and progression of heart failure, Congest. Heart Fail. 8 (2002) 132–140.
[34] P. Pacher, R. Schulz, L. Liaudet, C. Szabo, Nitrosative stress and pharmacological modulation of heart failure, Trends Pharmacol. Sci. 26 (2005) 302–310.
[35] Z. Ungvari, S.A. Gupte, F.A. Recchia, S. Batkai, P. Pacher, Role of oxidative-nitrosative stress and downstream pathways in various forms of cardiomyopathy and heart failure, Curr. Vasc. Pharmacol. 3 (2005) 221–229.
[36] A. Daiber, M. Oelze, M. Coldewey, K. Kaiser, C. Huth, S. Schildknecht, M. Bachschmid, Y. Nazirisade, V. Ullrich, A. Mulsch, T. Munzel, N. Tsilimingas, Hydralazine is a powerful inhibitor of peroxynitrite formation as a possible explanation for its beneficial effects on prognosis in patients with congestive heart failure, Biochem. Biophys. Res. Commun. 338 (2005) 1865–1874.
[37] J.M. Li, A.M. Shah, Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology, Am. J. Physiol., Regul. Integr. Comp. Physiol. 287 (2004) R1014–R1030.
[38] U.E. Ekelund, R.W. Harrison, O. Shokek, R.N. Thakkar, R.S. Tunin, H. Senzaki, D.A. Kass, E. Marban, J.M. Hare, Intravenous allopurinol decreases myocardial oxygen consumption and increases mechanical efficiency in dogs with pacing-induced heart failure, Circ. Res. 85 (1999) 437–445.
[39] J.W. de Jong, R.G. Schoemaker, R. de Jonge, P. Bernocchi, E. Keijzer, R. Harrison, H.S. Sharma, C. Ceconi, Enhanced expression and activity of xanthine oxidoreductase in the failing heart, J. Mol. Cell. Cardiol. 32 (2000) 2083–2089.
[40] T.P. Cappola, D.A. Kass, G.S. Nelson, R.D. Berger, G.O. Rosa, Z.A. Kobeissi, E. Marban, J.M. Hare, Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy, Circulation 104 (2001) 2407–2411.
[41] W.F. Saavedra, N. Paolocci, M.E. St John, M.W. Skaf, G.C. Stewart, J.S. Xie, R.W. Harrison, J. Zeichner, D. Mudrick, E. Marban, D.A. Kass, J.M. Hare, Imbalance between xanthine oxidase and nitric oxide synthase signaling pathways underlies mechanoenergetic uncoupling in the failing heart, Circ. Res. 90 (2002) 297–304.
[42] J.D. Lambeth, NOX enzymes and the biology of reactive oxygen, Nat. Rev. Immunol. 4 (2004) 181–189.
[43] C. Heymes, J.K. Bendall, P. Ratajczak, A.C. Cave, J.L. Samuel, G. Hasenfuss, A.M. Shah, Increased myocardial NADPH oxidase activity in human heart failure, J. Am. Coll. Cardiol. 41 (2003) 2164–2171.
[44] J.M. Li, A.M. Shah, Mechanism of endothelial cell NADPH oxidase activation by angiotensin II. Role of the p47phox subunit, J . Biol. Chem. 278 (2003) 12094–12100.
[45] N. Duerrschmidt, N. Wippich, W. Goettsch, H.J. Broemme, H. Morawietz, Endothelin-1 induces NAD(P)H oxidase in human endothelial cells, Biochem. Biophys. Res. Commun. 269 (2000) 713–717.
[46] L. Xiao, D.R. Pimentel, J. Wang, K. Singh, W.S. Colucci, D.B. Sawyer, Role of reactive oxygen species and NAD(P)H oxidase in alpha(1)-adrenoceptor signaling in adult rat cardiac myocytes, Am. J. Physiol., Cell Physiol. 282 (2002) C926–C934.
[47] A. Modesti, I. Bertolozzi, T. Gamberi, M. Marchetta, C. Lumachi, M. Coppo, F. Moroni, T. Toscano, G. Lucchese, G.F. Gensini, P.A. Modesti, Hyperglycemia activates JAK2 signaling pathway in human failing myocytes via angiotensin II-mediated oxidative stress, Diabetes 54 (2005) 394–401.
[48] J. Palomeque, L. Sapia, R.J. Hajjar, A. Mattiazzi, M.G. Vila Petroff, Angiotensin II-induced negative inotropy in rat ventricular myocytes: role of reactive oxygen species and p38 MAPK, Am. J. Physiol, Heart Circ. Physiol. 290 (2006) H96–H106.
[49] J.M. Li, A.M. Mullen, S. Yun, F. Wientjes, G.Y. Brouns, A.J. Thrasher, A.M. Shah, Essential role of the NADPH oxidase subunit p47(phox) in endothelial cell superoxide production in response to phorbol ester and tumor necrosis factor-alpha, Circ. Res. 90 (2002) 143–150.
[50] H. Matsushita, K. Lee, P.S. Tsao, Cyclic strain induces reactive oxygen species production via an endothelial NAD(P)H oxidase, J. Cell. Biochem. 81 (2001) 99–106.
[51] J.S. McNally, M.E. Davis, D.P. Giddens, A. Saha, J. Hwang, S. Dikalov, H. Jo, D.G. Harrison, Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress, Am. J. Physiol, Heart Circ. Physiol. 285 (2003) H2290–H2297.
[52] U. Landmesser, S. Dikalov, S.R. Price, L. McCann, T. Fukai, S.M. Holland, W.E. Mitch, D.G. Harrison, Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension, J. Clin. Invest. 111 (2003) 1201–1209.
[53] K.K. Griendling, D. Sorescu, M. Ushio-Fukai, NAD(P)H oxidase: role in cardiovascular biology and disease, Circ. Res. 86 (2000) 494–501.

[54] G.M. Kuster, D.R. Pimentel, T. Adachi, Y. Ido, D.A. Brenner, R.A. Cohen, R. Liao, D.A. Siwik, W.S. Colucci, Alpha-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes is mediated via thioredoxin-1-sensitive oxidative modification of thiols on Ras, Circulation 111 (2005) 1192–1198.
[55] L. Xiao, D.R. Pimental, J.K. Amin, K. Singh, D.B. Sawyer, W.S. Colucci, MEK1/2-ERK1/2 mediates alpha1-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes, J. Mol. Cell. Cardiol. 33 (2001) 779–787.
[56] K. Grote, I. Flach, M. Luchtefeld, E. Akin, S.M. Holland, H. Drexler, B. Schieffer, Mechanical stretch enhances mRNA expression and proenzyme release of matrix metalloproteinase-2 (MMP-2) via NAD(P)H oxidase-derived reactive oxygen species, Circ. Res. 92 (2003) e80–e86.
[57] W.K. Alderton, C.E. Cooper, R.G. Knowles, Nitric oxide synthases: structure, function and inhibition, Biochem. J. 357 (2001) 593–615.
[58] D.J. Stuehr, Mammalian nitric oxide synthases, Biochim. Biophys. Acta 1411 (1999) 217–230.
[59] C. Giulivi, J.J. Poderoso, A. Boveris, Production of NO by mitochondria, J. Biol. Chem. 273 (1998) 11038–11043.
[60] P. Ghafourifar, U. Schenk, S.D. Klein, C. Richter, Mitochondrial nitric-oxide synthase stimulation causes cytochrome *c* release from isolated mitochondria-evidence for intramitochondrial peroxynitrite formation, J. Biol. Chem. 274 (1999) 31185–31188.
[61] A.J. Kanai, L.L. Pearce, P.R. Clemens, L.A. Birder, M.M. VanBibber, S.Y. Choi, W.C. de Groat, J. Peterson, Identification of a neuronal nitric oxide synthase in isolated cardiac mitochondria using electrochemical detection, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 14126–14131.
[62] T. Michel, O. Feron, Nitric oxide synthases: which, where, how, and why? J. Clin. Invest. 100 (1997) 2146–2152.
[63] K.Y. Xu, D.L. Huso, T.M. Dawson, D.S. Bredt, L.C. Becker, Nitric oxide synthase in cardiac sarcoplasmic reticulum, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 657–662.
[64] L.J. Ignarro, Nitric Oxide: Biology and Pathobiology, Academic Press, San Diego, 2000.
[65] A. Boveris, L.E. Costa, J.J. Poderoso, M.C. Carreras, E. Cadenas, Regulation of mitochondrial respiration by oxygen and nitric oxide, Ann. N.Y. Acad. Sci. 899 (2000) 121–135.
[66] M.G. Petroff, S.H. Kim, S. Pepe, C. Dessy, E. Marban, J.L. Balligand, S.J. Sollott, Endogenous nitric oxide mechanisms mediate the stretch dependence of Ca2+ release in cardiomyocytes, Nat. Cell Biol. 3 (2001) 867–873.
[67] J.M. Hare, Nitric oxide and excitation–contraction coupling, J. Mol. Cell. Cardiol. 35 (2003) 719–729.
[68] G.C. Brown, C.E. Cooper, Nanomolar concentrations of NO reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase, FEBS Lett. 356 (1994) 295–298.
[69] G.C. Brown, V. Borutaite, Nitric oxide, cytochrome *c* and mitochondria, Biochem. Soc. Symp. 66 (1999) 17–25.
[70] G.C. Brown, Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome oxidase, Biochim. Biophys. Acta 1504 (2001) 46–57.
[71] D.J. Pinsky, S. Patton, S. Mesaros, V. Brovkovych, E. Kubaszewski, S. Grunfeld, T. Malinski, Mechanical transduction of nitric oxide synthesis in the beating heart, Circ. Res. 81 (1997) 372–379.
[72] M. Schweizer, C. Richter, NO potently and reversibly de-energizes mitochondria at low oxygen tension, Biochim. Biophys. Acta 204 (1994) 169–175.
[73] J.J. Poderoso, M.C. Carreras, C. Lisdero, N. Riobo, F. Schopfer, A. Boveris, NO inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles, Arch. Biochem. Biophys. 328 (1996) 85–92.
[74] R. Radi, M. Rodriguez, L. Castro, R. Telleri, Inhibition of mitochondrial electron transport by peroxynitrite, Arch. Biochem. Biophys. 308 (1994) 89–95.
[75] R. Bolli, Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research, J. Mol. Cell. Cardiol. 33 (2001) 1897–1918.
[76] G.C. Brown, V. Borutaite, Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols, Biochim. Biophys. Acta 1658 (2004) 44–49.
[77] G.C. Brown, Reversible binding and inhibition of catalase by nitric oxide, Eur. J. Biochem. 232 (1995) 188–191.
[78] F.R. Gadelha, L. Thomson, M.M. Fagian, A.D.T. Costa, R. Radi, A.E. Vercesi, Calcium-independent permeabilization of the inner mitochondrial membrane by peroxynitrite is mediated by membrane protein thiol cross-linking and lipid peroxidation, Arch. Biochem. Biophys. 345 (1997) 243–250.
[79] M. Estonius, O. Danielsson, C. Karlsson, H. Persson, H. Jornvall, J.O. Hoog, Distribution of alcohol and sorbitol dehydrogenases. Assessment of mRNA species in mammalian tissues, Eur. J. Biochem. 215 (1993) 497–503.
[80] L. Liu, A. Hausladen, M. Zeng, L. Que, J. Heitman, J.S. Stamler, A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans, Nature 410 (2001) 490–494.
[81] J.P. Kolb, Mechanisms involved in the pro- and anti-apoptotic role of NO in human leukemia, Leukemia 14 (2000) 1685–1694.
[82] P.S. Brookes, E.P. Salinas, K. Darley-Usmar, J.P. Eiserich, B.A. Freeman, V.M. Darley-Usmar, Concentration-dependent effects of nitric oxide on mitochondrial permeability transition and cytochrome *c* release, J. Biol. Chem. 275 (2000) 20474–20479.
[83] T. Sugamori, Y. Ishibashi, T. Shimada, N. Takahashi, T. Sakane, S. Ohata, Y. Kunizawa, S. Inoue, K. Nakamura, Y. Ohta, H. Shimizu, H. Katoh, N. Oyake, Y. Murakami, M. Hashimoto, Increased nitric oxide in proportion to the severity of heart failure in patients with dilated cardiomyopathy: close correlation of tumor necrosis factor-alpha with systemic and local production of nitric oxide, Circ. J. 66 (2002) 627–632.
[84] D.J. Ing, J. Zang, V.J. Dzau, K.A. Webster, N.H. Bishopric, Modulation of cytokine-induced cardiac myocyte apoptosis by nitric oxide, bak, and bcl-x, Circ. Res. 84 (1999) 21–33.
[85] I.H. Chae, K.W. Park, H.S. Kim, B.H. Oh, Nitric oxide induced apoptosis is mediated by Bax/Bcl-2 gene expression, transition of cytochrome *c*, and activation of caspase-3 in rat vascular smooth muscle cells, Clin. Chim. Acta 341 (2004) 83–91.
[86] G. Olivetti, R. Abbi, F. Quaini, J. Kajstura, W. Cheng, J.A. Nitahara, E. Quaini, C. Di Loreto, C.A. Beltrami, S. Krajewski, J.C. Reed, P. Anversa, Apoptosis in the failing human heart, N. Engl. J. Med. 336 (1997) 1131–1141.
[87] J. Narula, N. Haider, R. Virmani, T.G. DiSalvo, F.D. Kolodgie, R.J. Hajjar, Apoptosis in myocytes in end-stage heart failure, N. Engl. J. Med. 335 (1996) 1182–1189.
[88] D. Wencker, M. Chandra, K. Nguyen, W. Miao, S. Garantziotis, S.M. Factor, J. Shirani, R.C. Armstrong, R.N. Kitsis, A mechanistic role for cardiac myocyte apoptosis in heart failure, J. Clin. Invest. 111 (2003) 1497–1504.
[89] Q. Feng, X. Lu, D.L. Jones, J. Shen, J.M. Arnold, Increased inducible nitric oxide synthase expression contributes to myocardial dysfunction and higher mortality after myocardial infarction in mice, Circulation 104 (2001) 700–704.
[90] F. Sam, D.B. Sawyer, Z. Xie, D.L. Chang, S. Ngoy, D.A. Brenner, D.A. Siwik, K. Singh, C.S. Apstein, W.S. Colucci, Mice lacking inducible nitric oxide synthase have improved left ventricular contractile function and reduced apoptotic cell death late after myocardial infarction, Circ. Res. 89 (2001) 351–356.
[91] M.J. Szabolcs, S. Ravalli, O. Minanov, R.R. Sciacca, R.E. Michler, P.J. Cannon, Apoptosis and increased expression of inducible nitric oxide synthase in human allograft rejection, Transplantation 65 (1998) 804–812.
[92] B.S. Kristal, J. Chen, B.P. Yu, Sensitivity of Mitochondrial Transcription to Different Free Radical Species, Free Radic. Biol. Med. 16 (1994) 323–329.
[93] S. Srivastava, B. Chandrasekar, A. Bhatnagar, S.D. Prabhu, Lipid peroxidation-derived aldehydes and oxidative stress in the failing heart: role of aldose reductase, Am. J. Physiol. 283 (2002) H2612–H2619.
[94] M. Kaneko, R.E. Beamish, N.S. Dhalla, Depression of heart sarcolemmal Ca2+ pump activity by oxygen free radicals, Am. J. Physiol. 256 (1989) H368–H374.

[95] M. Kim, T. Akera, O2 free radicals: cause of ischemia–reperfusion injury to cardiac Na+-K+-ATPase, Am. J. Physiol. 252 (1987) H252–H257.
[96] P.S. Haddock, M.J. Shattock, D.J. Hearse, Modulation of cardiac Na+–K+ pump current: role of protein and non-protein sulfhydryl redox status, Am. J. Physiol. 269 (1995) H297–H307.
[97] E.J. McConnell, A.M. Bittelmeyer, B.U. Raess, Irreversible Inhibition of Plasma membrane (Ca2+ + Mg2+)-ATPase and Ca2+ transport by 4-OH-2,3-*trans*-nonenal, Arch. Biochem. Biophys. 361 (1999) 252–256.
[98] J.I. Goldhaber, S. Ji, S.T. Lamp, J.N. Weiss, Effects of exogenous free radicals on electromechanical function and metabolism in isolated rabbit and guinea pig ventricle — implications for ischemia and reperfusion injury, J. Clin. Invest. 83 (1989) 1800–1809.
[99] J.L. Zweier, J.T. Flaherty, M.L. Weisfeldt, Direct measurement of free radical generation following reperfusion of ischemic myocardium, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 1404–1407.
[100] G. Ambrosio, J.L. Zweier, J.T. Flaherty, The relationship between oxygen radical generation and impairment of myocardial energy metabolism following post-ischemic reperfusion, J. Mol. Cell. Cardiol. 23 (1991) 1359–1374.
[101] G.M. Hatch, Cell biology of cardiac mitochondrial phospholipids, Biochem. Cell. Biol. 82 (2004) 99–112.
[102] T.H. Haines, N.A. Dencher, Cardiolipin: a proton trap for oxidative phosphorylation, FEBS Lett. 528 (2002) 35–39.
[103] G. Paradies, G. Petrosillo, M. Pistolese, F.M. Ruggiero, Reactive oxygen species affect mitochondrial electron transport complex I activity through oxidative cardiolipin damage, Gene 286 (2002) 135–141.
[104] G. Paradies, G. Petrosillo, M. Pistolese, N. Di Venosa, A. Federici, F.M. Ruggiero, Decrease in mitochondrial complex I activity in ischemic/reperfused rat heart—Involvement of reactive oxygen species and cardiolipin, Circ. Res. 94 (2004) 53–59.
[105] G. Paradies, G. Petrosillo, M. Pistolese, N. Di Venosa, D. Serena, F.M. Ruggiero, Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion, Free Radic. Biol. Med. 27 (1999) 42–50.
[106] F.L. Hoch, Cardiolipins and mitochondrial proton-selective leakage, J. Bioenerg. Biomembranes 30 (1998) 511–532.
[107] B. Hoffman, A. Stockl, M. Schlame, K. Beyer, M. Klingenberg, The reconstituted ADP/ATP carrier activity has an absolute requirement for cardiolipin as shown in cysteine mutants, J. Biol. Chem. 269 (1994) 1940–1944.
[108] M. Degli Esposti, Mitochondria in apoptosis: past, present and future, Biochem. Soc. Trans. 32 (2004) 493–495.
[109] G.C. Sparagna, C.A. Johnson, S.A. McCune, R.L. Moore, R.C. Murphy, Quantitation of cardiolipin molecular species in spontaneously hypertensive heart failure rats using electrospray ionization mass spectrometry, J. Lipid Res. 46 (2005) 1196–1204.
[110] K. Okumura, Y. Yamada, J. Kondo, H. Hashimoto, T. Ito, J. Kitoh, Decreased 1,2-diacylglycerol levels in myopathic hamster hearts during the development of heart failure, J. Mol. Cell. Cardiol. 23 (1991) 409–416.
[111] C. Richter, J. Park, B.N. Ames, Normal Oxidative Damage to Mitochondrial and Nuclear DNA is Extensive, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 6465–6467.
[112] T. Ide, H. Tsutsui, S. Hayashidani, D. Kang, N. Suematsu, K. Nakamura, H. Utsumi, N. Hamasaki, A. Takashita, Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction, Circ. Res. 88 (2001) 529–535.
[113] F. Rosenfeldt, D. Hilton, S. Pepe, H. Krum, Systematic review of effect of coenzyme Q10 in physical exercise, hypertension and heart failure, Biofactors 18 (2003) 91–100.
[114] C.H. Strey, J.M. Young, S.L. Molyneux, P.M. George, C.M. Florkowski, R.S. Scott, C.M. Frampton, Endothelium-ameliorating effects of statin therapy and coenzyme Q10 reductions in chronic heart failure, Atherosclerosis 179 (2005) 201–206.
[115] G.P. Littarru, L. Tiano, Clinical aspects of coenzyme Q10: an update, Curr. Opin. Clin. Nutr. Metab. Care 8 (2005) 641–646.
[116] A.K. Dhalla, M.F. Hill, P.K. Singal, Role of oxidative Stress in transition of hypertrophy to heart failure, J. Am. Coll. Cardiol. 28 (1996) 506–514.
[117] Author not listed, Vitamin E gets an F. Research linking high doses of vitamin E to heart failure is causing new worries about the AREDS vitamins for macular degeneration, Harv. Health Lett. 30 (2005) 6.
[118] L. Tavazzi, G. Tognoni, M.G. Franzosi, R. Latini, A.P. Maggioni, R. Marchioli, G.L. Nicolosi, M. Porcu, GISSI-HF Investigators, Rationale and design of the GISSI heart failure trial: a large trial to assess the effects of n-3 polyunsaturated fatty acids and rosuvastatin in symptomatic congestive heart failure, Eur. J. Heart Fail. 6 (2004) 635–641.
[119] S. Pepe, N. Tsuchiya, E.G. Lakatta, R.G. Hansford, PUFA and aging modulate cardiac mitochondrial membrane lipid composition and Ca2+ activation of PDH, Am. J. Physiol. 276 (1999) H149–H158.
[120] S. Pepe, P.L. McLennan, Cardiac membrane fatty acid composition modulates myocardial oxygen consumption and postischemic recovery of contractile function, Circulation 105 (2002) 2303–2308.
[121] S. Pepe, Effect of dietary polyunsaturated fatty acids on age-related changes in cardiac mitochondrial membranes, Exp. Gerontol. 40 (2005) 751–758.
[122] D. Mozaffarian, C.L. Bryson, R.N. Lemaitre, G.L. Burke, D.S. Siscovick, Fish intake and risk of incident heart failure, J. Am. Coll. Cardiol. 45 (2005) 2015–2021.
[123] M. Maytin, W.S. Colucci, Cardioprotection: A New Paradigm in the Management of Acute Heart Failure Syndromes, Am. J. Cardiol. 96 (2005) 26G–31G (suppl).
[124] K. Todaka, J. Wang, G.H. Yi, R. Stennett, M. Knecht, M. Packer, D. Burkhoff, Effects of Levosimendan on myocardial contractility and oxygen consumption, J. Pharmacol. Exp. Ther. 279 (1996) 120–127.
[125] T. Sorsa, P. Pollesello, R.J. Solaro, The contractile apparatus as a target for drugs against heart failure: interaction of levosimendan, a calcium sensitizer, with cardiac troponin C, Mol. Cell. Biochem. 266 (2004) 87–107.
[126] Z. Papp, K. Csapo, P. Pollesello, H. Haikala, I. Edes, Pharmacological mechanisms contributing to the clinical efficacy of levosimendan, Cardiovasc. Drug Rev. 23 (2005) 71–98.
[127] V.S. Moiseyev, P. Poder, N. Andrejevs, M.Y. Ruda, A.P. Golikov, L.B. Lazebnik, Z.D. Kobalava, L.A. Lehtonen, T. Laine, M.S. Nieminen, K.I. Lie, on behalf on the RUSSLAN Study Investigators, Safety and efficacy of a novel calcium sensitizer, levosimendan, in patients with left ventricular failure due to an acute myocardial infarction: a randomized, placebo-controlled double-blind study (RUSSLAN), Eur. Heart J. 23 (2002) 1422–1432.
[128] H. Ono, T. Osanai, H. Ishizaka, H. Hanada, T. Kamada, H. Onodera, N. Fujita, S. Sasaki, T. Matsunaga, K. Okumura, Nicorandil improves cardiac function and clinical outcome in patients with acute myocardial infarction undergoing primary percutaneous coronary intervention: role of inhibitory effect on reactive oxygen species formation, Am. Heart J. 148 (2004) 1–7.
[129] J.N. Cohn, G.R. Johnson, R. Shabetai, H. Loeb, F. Tristani, T. Rector, R. Smith, R. Fletcher, Ejection fraction, peak exercise oxygen consumption, cardiothoracic ratio, ventricular arrhythmias, and plasma norepinephrine as determinants of prognosis in heart failure. The V-HeFT VA Cooperative Studies Group, Circulation 87 (1993) VI5–VI16 (Suppl.).
[130] K. Nakamura, K. Kusano, Y. Nakamura, M. Kakishita, K. Ohta, S. Nagase, M. Yamamoto, K. Miyaji, H. Saito, H. Morita, T. Emori, H. Matsubara, S. Toyokuni, T. Ohe, Carvedilol decreases elevated oxidative stress in human failing myocardium, Circulation 105 (2002) 2867–2871.